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Margaret P. Drosos

APPLICATION TRANSMITTAL LETTER
37 C.F.R. § 1.53(b)(1) AND (d)(1)

Box PATENT APPLICATION
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Dear Sir:

Transmitted herewith for filing is the patent application under 37 C.F.R. §1.53(b)(1) and (d)(1) of Linda A. SHERMAN and Joseph LUSTGARTEN for RECOMBINANT CONSTRUCTS ENCODING T CELL RECEPTORS SPECIFIC FOR HUMAN HLA-RESTRICTED TUMOR ANTIGENS.

Enclosed are:

- ☒ 14 Pages of specification.
- ☒ 4 Pages of claims.
- ☒ 1 Pages of abstract.
- ☒ 12 Sheet(s) of drawing(s).
- ☒ Combined Declaration and Power of Attorney of the inventors [unsigned].
- ☐ Power of Attorney and Prosecution by Assignee under 37 C.F.R. § 3.71.
- ☐ Assignment.
- ☐ Assignment Recordation Form.
- ☐ Computer program in microfiche.
- ☐ Verified statement(s) claiming small entity status.
- ☐ Other:

The filing fee has been calculated as follows:

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TOTAL CLAIMS	21 - 20 =	1	x $22.00	$22.00
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MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $260.00	$ 00.00
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RECOMBINANT CONSTRUCTS ENCODING T CELL RECEPTORS SPECIFIC FOR HUMAN HLA-RESTRICTED TUMOR ANTIGENS

Technical Field

The invention is directed to recombinant T cell receptors and modified forms thereof that are useful in identifying displayed tumor antigens and in antitumor therapy.

Background Art

Cytotoxic T lymphocytes (CTLs) form an essential part of an immune response to infectious agents and to malignancies. Thus, CTLs which are directed to established tumors may be effective in destroying these targets. Greenberg, P.D. *Adv Immunol* (1991) 49:281-355. CTL may also be used to identify tumor-specific antigens such as MAGE, GP100, tyrosinase, and MART, as well as broadly expressed tumor-associated antigens such as P53 (Yanuck, M. *et al. Cancer Res* (1993) 53:3257-3261) ; Houviers, J.G.A. *et al. Eur J Immunol* (1993) 23:2072-2077; Her-2/neu (Peoples, G.E. *et al. Proc Natl Acad Sci USA* (1995) 92:432-436; Fisk, B. *et al. J Exp Med* (1995) 181:2109-2177; as well as the tumor antigen Ras (Skipper, J. *et al. J Exp Med* (1993) 177:1493-1498).

It has been typical that such tumor-specific CTLs have been obtained from tumor infiltrating lymphocytes (TILs). However, this is subject to a number of disadvantages due to the complexity of the system and the endogenous mechanisms to counteract the effect of these CTLs. Importantly, the most effective CTLs may have been eliminated (Schwartz, R.H. *Cell* (1989) 57:1073-1081); the target tumors may have become resistant (Browning, M.J. *et al. Curr Opin Immunol* (1992) 4:613-618); or the T cells may lose functional activity by down-regulating expression of the ζ chain of the CD3 complex or the p^{56} LCK molecules (Mizoguchi, H. *et al. Science* (1992) 258:1795-1798).

In order to overcome these disadvantages, the present applicants have used transgenic mice as a source of CTLs that contain the desired nucleotide sequences

encoding TCRs specific for tumor-associated antigens restricted by human HLAs. Both humans and HLA-A2 transgenic mice select the same A2-restricted antigenic epitopes from influenza (Vitiello, A. *et al. J Exp Med* (1991) 173:1007-1015). Also, the present applicants have shown that HLA-A2 transgenic mice can produce p53-specific, A2 restricted CTLs when immunized with certain p53 derived peptides. Theobald, M. *et al.*. *Proc Natl Acad Sci USA* (1995) 92:11993-11997.

Of course, if murine-derived TCRs are to be used in a human context, humanization of such TCRs would be advantageous. In order to avoid competition for dimerization with endogenous Vα/Cα or Vβ/Cβ TCR, it may be advantageous to prepare chimeric TCRs using the ζ region of the CD3 receptor as the transmembrane and cytoplasmic domain. Such constructs could be prepared in either dimeric or single-chain form. Competition by Vα/Cα or Vβ/Cβ for each other or for the availability of CD3 chains has already been shown by Gorochov, *International J Cancer* (1992) 8:53-57 and by Wegener, A.M.K. *et al. Cell* (1992) 68:83. Chimeric Vα/ζ + Vβ/ζ chimeras were described by Engel, I. *et al. Science* (1992) 256:1318 who also showed that such chimeras could be activated by exposure to the appropriate antigen-MHC complex. In addition, Irving, B.A. *et al. Cell* (1991) 64:891 reported that chimeric molecules composed of the CD8/ζ or CD16/ζ and expressed in T cells had the capacity to transduce activation signals for IL-2 production and mediated specific cell lysis in a manner indistinguishable from those generated by the TCR/CD3 complex. In addition, Chung, S. *et al. Proc Natl Acad Sci USA* (1994) 91:12654-12658 constructed a single-chain TCR (scTCR) using the ζ-chain of CD3 and expressed it in T cells, thus conferring the T cells with the relevant specificity. These T cells further produce IL-2 on activation with the specific antigen. The present applicants have further confirmed this approach using clone 4 TCR as a model system.

However, there remains a need for a convenient source of nucleic acids encoding TCR molecules and their modified forms which are human HLA restricted and specific for common tumor-associated antigens. The present invention supplies this need.

Disclosure of the Invention

The invention provides materials that are useful in tumor diagnosis and therapy by permitting altered T lymphocytes to recognize and destroy unwanted tumor tissue. T cell receptor-encoding nucleic acid molecules can be obtained by immunizing transgenic mice which produce human HLA with tumor-associated antigens and recovering the nucleic acids encoding the T cell receptors from the cytotoxic T lymphocytes (CTL).

Thus, in one aspect, the invention relates to a method to prepare an isolated nucleic acid molecule comprising a nucleotide sequence encoding at least one of the variable regions of the α and β chains of a non-human TCR which TCR is human HLA-restricted and specific for a tumor-associated antigen, which method comprises cloning or amplifying a nucleic acid molecule containing said encoding nucleotide sequence from the CTL prepared by a method which comprises immunizing a transgenic non-human vertebrate which is modified so as to express at least one human HLA antigen with said tumor-associated antigen (TAA) so as to effect the production in said mouse of cytotoxic T lymphocytes which display human HLA-restricted TCR specific for said TAA and which contain nucleic acid molecules comprising nucleotide sequences encoding the α and β chain of said TCR and recovering the CTL.

In other aspects, the invention relates to nucleic acid molecules obtained by the foregoing method and to constructs employing their variable regions, to cells displaying TCRs or derivatives encoded by said nucleic acids or their modified forms, and use of these materials in diagnosis and therapy of human tumors.

Brief Description of the Drawings

Figure 1 shows the structure of several derivatives of effective T cell receptors wherein the ζ region is substituted as a chimeric transmembrane and cytoplasmic region.

Figure 2 shows, in more detail, the construction of the nucleotide sequence encoding such derivatives.

Figure 3 shows the complete nucleotide sequence and deduced amino acid sequence of a single chain TCR derivative which contains variable α and β specific for HA linked through a short peptide linker and then fused through a CD8 hinge to the ζ chain.

Figure 4 shows the ability of cells transfected with various modified TCR forms specific for HA to produce IL2 in response to stimulation with HA.

Figure 5 shows the ability of CTL's generated in mice in response to Her 2/neu-peptides H3 and H7 to mice H7 or H3 bearing targets. CTLs from both A2.1xK^{b}xCD8 and from A2.1 transgenic mice were comparable in result.

Figure 6 shows the sequence of various primers useful in cloning or amplifying the nucleotide sequences in coding during variable regions of α and β TCR chains.

Figures 7A and 7B show the nucleotide sequence and deduced amino acid sequence of the variable regions of the α and β chains of H7-specific TCR respectively.

Figure 8 shows a diagram of an expression vector suitable for producing the modified TCRs of the invention.

Figure 9 shows the ability of H7 specific modified TCR forms transfected in the 27J cells to effect IL2 production in said cells in response to the H7 peptide when the H7 peptide is presented in the presence of JA2 cells.

Figure 10 shows the ability of the various modified H7 specific TCR constructs to stimulate IL2 production in 27J cells in response to tissues bearing Her2/neu-peptides.

Modes of Carrying Out the Invention

The invention provides a convenient source for desirable recombinant materials that are useful in therapeutic and diagnostic procedures related to human tumors. Specifically, the materials of the invention provide a means whereby enhanced populations of cells that display appropriate TCRs for identifying and destroying tumor tissue may be obtained, as well as providing cells that are useful in evaluating the tumor-associated antigen that could usefully be targeted.

Briefly, the recombinant materials are obtained from CTL produced by immunizing nonhuman subjects with tumor-associated antigens associated with human tumors, where the nonhuman subject has been modified so as to be capable of expressing a human HLA. Thus, the relevant TCRs are not only specific for the human tumor-associated antigen, but also restricted by a human HLA. While murine subjects are clearly the most convenient at the present time, further developments in the construction of transgenic animals may permit alternative nonhuman subjects to be used equally conveniently in the near future. Such additional nonhuman subjects may include rats, avian subjects, larger mammals, or any appropriate vertebrate system that can be manipulated to provide it with human HLA and which can mount an immune response to provide CTLs with the appropriate T cell receptors.

Further, while the human HLA illustrated herein is A2, there is no theoretical reason why other HLA domains such as A1, A3, and B7 could not be used as well. Because transgenic mice are readily available which produce this antigen, the use of a A2 as the restrictive antigen is simply a matter of convenience. In addition, if murine subjects are used, and the MHC region is entirely human, it is preferred to use mice transgenic so as to express human CD8 as well as human Class MHC antigen. This is due to the inability of murine CD8 to interact effectively with human A2.1. Thus, expression of human CD8 on the murine cells facilitates lysis of target antigen presenting cells. On the other hand, for mice transgenic for MHC human/mouse chimeras, such as $A2K^b$ mice also examplified below, the presence of human CD8 is not necessary.

The recombinant materials relevant to the invention include those associated with the TCR produced by the nonhuman subject *per se*, and also derivatives of this TCR which retain their HLA restriction and specificity characteristics. Such derivatives contain the variable regions of the α and β chains either as dimers or in single chain form and are more advantageous than the nonhuman TCR *per se* for a number of reasons. First, if the desired TCR can be "humanized," less unwanted side-reactions can be expected. Second, economies of production can be effected if shorter peptides can be substituted for the TCR

per se. Third, if the TCR is produced as a single chain, rather than in its customary dimeric form, economies of production and ease of association of the relevant variable units are achieved. In all cases, substituting a derivative for one or both of the α and β chains or a single-chain form containing variable regions of both α and β precludes the formation of hybrid TCRs wherein for example the desired TCR α chain is coupled with an endogenous TCR β. Thus, the recovery of cells which produce the desired derivative is greater.

Figures 1 and 2 describe some typical derivatives of TCRs useful in the invention. As shown in Figure 1, a dimeric form may be constructed wherein the variable regions of both α and β chains are directly coupled to the ζ regions of various CD receptors such as CD3, CD8 and CD16. These ζ regions substitute for the transmembrane and cytoplasmic regions normally associated with the TCR. In these examples, the constant region, as it is unnecessary, is eliminated in any case.

Further, in Figure 1, an alternative construction includes a CD8 hinge region between the variable region and the transmembrane portion of the ζ chain. This spacer may assist in appropriate folding of the receptor. Similarly, in Figure 1, construction of a single chain TCR wherein the variable regions of the α and β chains are fused through a linker and then fused to the ζ region is shown with and without the CD8 hinge.

Figure 2 shows a pattern for construction of the relevant plasmids containing the nucleotide sequences encoding the derivatives shown in Figure 1. As shown hereinbelow, a model system wherein clone 4 TCR directed against hemaglutinin antigen (HA) was used to supply the variable region verified the operability of these approaches.

It is important to recognize that the critical feature of the nucleic acid encoding the TCR derivative is the presence of the variable regions from the α and β chains, and that additional sequence, perhaps for added stability, including some or all of the constant region may be present. In addition, alternative transmembrane and signalling regions other than the ζ regions examplified above may be substituted. Thus, the recombinant materials encoding the TAA-specific, human MHC restricted TCR derivatives of the invention need

only include the variable α and β regions of the relevant TCR along with some additional transmembrane and signalling sequence and may further include additional non-interfering amino acid sequence.

The desired CTLs will be specific for TAAs associated with human cancers. Typical among these is Her-2/neu since this proto-oncogene is overexpressed in many human cancers and associated with aggressive disease and malignant transformation (Press, M.S. *et al. Cancer Res* (1994) 54:5675-5682; Slamon, D. *et al. Science* (1987) 235:177-182). Other suitable tumor-associated antigens include Ras, p53, tyranase, MART, Gp100, MAGE, BAGE and MUC-1. Any desired antigen which is associated with human tumors can readily be used.

The availability of nucleic acid molecules encoding the desired TCR permits of both diagnostic and therapeutic uses. Cells displaying the TCR at their surfaces can be used as diagnostic for the TAA that is actually expressed by the tumor. In order to conduct such assays, the tumor or a portion thereof or cells derived therefrom are exposed to cells transfected to contain an expression system for the TCR or derivative and the ability of the recombinant CTLs to lyse the tumor cells is assessed. The procedure described in Theobald, M., *et al.* (1995) *supra*, may, for example, be used.
In addition, an expression for the appropriate TCR may be used therapeutically by transducing such an expression system into the peripheral blood lymphocytes (PBL) $CD8^+$ T cells from a tumor-bearing host via, for example, retroviral-mediated gene transfer. Such transfer techniques are known in the art. See, for example, Kasid, A. *et al. Proc Natl Acad Sci USA* (1990) 87:473, Rosenberg, S.A. *et al. New England Journal of Medicine* (1990) 323:570. The altered $CD8^+$ cells then provide a passive form of immunotherapy. Of course, humanized forms of the TCR as the appropriate derivatives are most helpful in this application.

The following examples are intended to illustrate but not to limit the invention.

Preparation A

Model System for TCR Derivatives

Clone 4 TCR (reference) is specific for the hemaglutinin antigen (HA). As the nucleotide sequences encoding the α and β chains of this TCR are available, constructs were made to mimic the intended derivatives of the TAA-specific, HLA-restricted TCR of the invention.

Briefly, four types of chimeric molecules were constructed: two are the dimers obtained as α/ζ + the β/ζ and two are single-chain TCR/ζ chimeric molecules analogous to those shown in Figure 1 herein. The complete nucleotide sequence encoding the single chain form with the CD8 hinge is shown in Figures 3A-3B. These four constructs were transfected into the T cell hybridoma MD.45-27 and the transformants were grown under neomycin selection and screened for IL-2 secretion upon stimulation with either spleen cells from Balb/c or P815(H-2^d) cells pulsed with the HA-specific peptide or RENCA tumor cell line transfected with the HA gene. The results showing the levels of IL-2 produced are shown in Figure 4. As shown, none of the transfectants showed appreciable production of IL-2 in the absence of HA. Only the transfectants containing the clone 4 derivatives showed stimulation of IL-2 production when HA was present. Both single-chain forms, with and without the CD8 hinge and both dimeric forms, both with and without the CD8 hinge showed appreciable stimulation of IL-2 production when treated either with Balb/c spleen cells plus HA peptide, P815 cells plus HA peptide, or RENCA cells expressing HA at their surfaces.

Example 1

Selection of Her-2/neu Immunogenic Peptides

Eighteen peptides were synthesized based on the sequence of the human Her-2/neu protein wherein each sequence contained the anchor motif for HLA A2.1, that is, L, I, M, V, A, T at position 2 and position 8/9/10 (Rupert, J. *et al. Cell* (1993) 74:929-937). The binding efficiency of these peptides to A2 was determined using a competition assay as

described by Morrison, J. *et al. Eur J Immunol* (1992) 22:903-907. Briefly, each test peptide (10 μg) was incubated with radiolabeled target cells (T2-A2.1/K^b, 10^6 target cells labeled with 150 μg ^{51}Cr at 37° for 1.5 hours) in the presence of an influenza virus matrix protein (0.1 μg). The ability of these peptides to inhibit the binding of the influenza matrix protein peptide M1 (58-66) to A2.1 was measured by inhibition of lysis by an M1 (58-66) specific, A2.1 restricted CTL clone. As shown in Table 1, many of the tested peptides were able to inhibit binding of the M1 peptide.

Table 1. Her-2/neu peptides used for immunization

PEPTIDE	SEQUENCE #	SEQUENCE	IMMUNOGENICITY	% INHIBITION
H3	369-377	KIFGSLAFL	+	38
H6	444-453	TLQGLGISWL	-	56
H7	773-782	VMAGVGSPYV	+	55
H8	546-555	VLQGLPREYV	-	43
H12	48-56	HLYQGQQW	-	15
H13	689-697	RLLQETELV	-	56
H14	747-755	KIPVAIKVL	-	35
H15	789-797	CLTSTVQLV	-	33
H16	799-807	QLMPYGCLL	-	50
H17	851-859	VLVKSPNHV	-	12
H18	871-879	DIDETEYHA	-	37
H19	933-941	DLLEKGERL	-	36
H20	971-979	ELVSEFSRM	-	5
H21	971-980	ELVSEFSRMA	-	25
H22	972-980	LVSEFSRMA	-	14
H23	1016-1024	DLVDAEEYL	-	35
H24	1172-1180	TLSPGKNGV	-	57
HIV-9K	POL	KLVGKLNWA	+	80

The peptides were then tested for their ability to elicit an immune response *in vivo*. The peptides were administered either to A2.1/K^bxCD8 or A2.1 transgenic mice and primary cultures of CTLs were generated. Mice were immunized with a mixture of 100μg of the Her-2/neu peptide with 120μg 'helper' peptide (the helper peptide is a I-A^b restricted peptide derived from Hepatitis B virus core protein comprising amino acid

residues 128 to 140, that induces a strong CD4 helper response) in 100µl Incomplete Freuhd's adjuvant. A2.1/K^bxCD8 lipopolysacharide (LPS)-blasts were prepared as stimulators for *in vitro* restimulation of spleen cells from immunized mice. These were prepared by incubating splenocytes in complete RPMI containing 25 µg/ml LPS and 7 µg/ml dextran sulfate at $1.5x10^6$ cells/ml in a total volume of 30 ml for 3 days. Murine spleen cells, collected 10 days after immunization, were restimulated *in vitro* with the irradiated (3000rads) blasts which had bound Her-2/neu specific peptides. Six days following *in vitro* restimulation, the CTL populations were assayed for lytic activity against T2-A2.1/K^b target cells preincubated with the peptide used for stimulation (15µM). The resultant Her-2/neu peptide-specific CTL populations were maintained *in vitro* by weekly restimulation. CTL populations were restimulated in 2ml cultures by incubating with 0.1-0.2 x 10^6 irradiated Jurkat-A2.1 cells (20,000 rad) preincubated with Her-2/neu peptide (15µM) and $5x10^5$ irradiated C57BL/6 spleen cells (3000 rad) as fillers in complete RPMI media containing 2% (v/v) supernatent from concanavalin A stimulated rate spleen cells (TCGF).

The cultured cells were assayed for cytotoxicity against T2A2.1/K^b target cells pulsed with the priming peptide. In the cytotoxicity assay, 10^6 target cells were incubated at 37°C with 150 µCi of sodium ^{51}Cr chromate for 90 minutes, in the presence or absence of specific peptide. Cells were washed three times and resuspended in 5% RPMI. For the assay, 10^4 ^{51}Cr-labeled target cells were incubated with different concentrations of effector cells in a final volume of 200µl in U-bottomed 96 well plates. Supernatants were removed after 4-7 hrs. at 37°C, and the percent specific lysis was determined by the formula: percent specific lysis = 100 x (experimental release-spontaneous release)/(maximum release-spontaneous release). As shown in Table 1, only the H3 and H7 peptides were able to stimulate a CTL response. (The HIV-9K peptide, known to be immunogenic, was used as a control.)

CTL populations that were specific for H3 and H7 were established from either murine strain and maintained *in vitro* by weekly restimulation. The results of testing these

established cell cultures for their ability to lyse T2-labeled targets at a ratio of 1:1 in a four-hour assay in the presence of peptide H3 or H7 are shown in Figure 5. As shown, the CTLs from either murine subject were comparably effective at comparable peptide concentrations.

Example 2

Lysis of Human Tumors by H3- and H7-Specific CTL

Various tumor cell lines were characterized by FACS analysis for surface expression of A2 and Her-2/neu peptides. These tumor cells and other control tumors were preincubated or not for 24 hours in media supplemented with 20 ng/ml γ-IFN and 3 ng/ml TNF-α, as such pretreatment increases expression of MHC-1 and adhesion molecules thus enhancing their sensitivity to lysis (Fady, C. *et al. Cancer Immuno Immunother* (1993) 37:329-336; Fisk, B. *et al. Lympho and Cytokine Res* (1994) 13:125-131). In the assay, the tumor cells were mixed with the H3- or H7-specific CTL for 6 hours and lysis was measured. HIV-9K-specific CTL were used as a control. The results are shown in Table 2.

Table 2. Killing of tumor expressing Her-2/neu

TUMOR	TYPE	A2	Her-2	H7	H7 + CYT	H3	H3 + CYT	HIV-9K	HIV-9K + CYT
MDA.MB231	BREAST	+	+	26	89	34	85	3	14
MCF-7	BREAST	+	+	7	40	7	54	3	7
BT549	BREAST	+	+	2	36	2	40	2	15
SAOS.175	OSTEOSARCOMA	+	+	27	35	27	33	18	11
U2-OS	OSTEOSARCOMA	+	+	30	62	32	91	18	24
SW480	COLON	+	+	2	17	6	50	1	4
OVCAR-5	OVARIAN	+	+	13	23	25	29	10	12
T98G	GLIOBLASTOMA	+	+	29	93	20	99	9	13
MALME-3M	MELANOMA	+	+	4	14	28	57	2	1
SKMEL-5	MELANOMA	+	+	16	40	6	38	5	4
NCI.H1355	LUNG	+	+	13	62	11	38	7	25
Hep-G2	HEPATOMA	+	+	4	29	4	20	1	8
CASKI	CERVIX	+	+	9	20	13	30	8	11
U87G	GLIOBLASTOMA	+	-	1	1	2	1	5	1
ST486	LYMPHOMA	+	-	5	8	1	1	1	1
LG-2	EBV-TRANS.	+	-	1	3	2	4	1	1
SV80	FIBROBLAST	+	-	2	2	4	8	2	2
JY	LYMPHOMA	+	-	4	2	2	1	2	1
MDA.MB435	BREAST	-	+	1	1	3	2	4	3

As shown, the CTLs were able to lyse effectively only those tumors expressing both A2 and Her-2 peptides. Further, repeating the experiment in the presence of an anti-A2 antibody significantly decreased lysis, and H3 and H7 could be extracted from the tumors using standard techniques.

In a manner similar to that set forth above with respect to H3 and H7, A2-restricted CTLs specific for p53 have been generated. Theobald, M. *et al.* (1995) (*supra*).

Example 3

Recovery of Genes Encoding Her-2/neu and p53 TCRs

The genes encoding the relevant α and β chains of the TCR specific for H3, H7, and p53 are cloned according to the method of Zisman, B. *et al. Eur J Immunol* (1994) 24:2497-2505. Primers for the PCR amplification according to these methods are derived from Vα or Vβ families paired with Cα or Cβ primer. Suitable primers for use in this process are shown in Figure 6. The amplified PCR products are cloned into Bluescript vectors and sequenced. Figure 7 shows the sequences of the variable regions of the α and

β chains of the TCRs recovered from CTLs recovered in mice that had been administered the H7 peptide.

Chimeric molecules similar to those described hereinabove for clone 4 and as set forth in Figures 1 and 2 were prepared from the amplified sequences of the H7-specific RR functionality is assayed by transfecting MD45.27 and testing for the production of IL-2 as described hereinabove.

A preferred vector for the insertion of the modified sequences, pBJ1Neo with a polylinker insertion site is shown in Figure 8. The host vector, pBJINeo is described in _____, *Mol Cell Biol* (1988) 8:466; the polylinker is described by _____, Science (1990) 249:677.

The dimer and single chain constructs were transfected into 27J cells and the cells measured for production of IL-2 in the presence of JA^2 cells plus H7 peptide. As shown in Figure 9, all transfectants produced with the H7 specific TCR derivatives produced IL-2. 27J cells without these constructs did not produce IL-2 in response to the JA2 cells and peptide, and none of the cells produced IL-2 in response to JA2 cells alone.

Finally, Figure 10 shows the production of IL-2 by these four constructs transfected into 27J cells in response to HER 2/neu derived peptides and cells presenting such peptides. Again, all four constructs rendered the transfected cells responsive.

Example 4

Preparation of T cells Expressing TCR and its Derivatives

Human PBL that are CD8+ are transduced with the chimeric constructs described above using the LXSN and LXSH retroviral vectors (Hock, R.A. *et al. Nature* (1986) 320:275) and the technique of Anderson, W.F. *Science* (1992) 256:808. The β chimeric gene is inserted into the LXSH retroviral vector which confers Hygromycin B resistance and α chimeric gene in LXSN retroviral vector which confers neomycin resistance; thus selection of T lymphocytes expressing both the Vα/ζ and Vβ/ζ can be recovered. Recombinant retrovirus-producing cell lines are generated by transfection of the vectors

into the Ecotropic packaging cell line GP+E86 and the ecotropic virus produced by these cells is used to infect the amphotropic packaging cell line PA317. PA317 clones that produce helper virus free from amphotropic L(Vα/ζ)SN and L(Vβ/ζ)SH virus are obtained by selection in G418 or Hygromycin B-containing medium. Clones yielding the highest titer of virus are used to transduce T lymphocytes that have been incubated with anti-CD3 and recombinant IL-2. Similarly, the single-chain TCR is inserted into LXSN retroviral vector and introduced similarly.

The resulting transformed human $CD8^+$-PBL are tested for cytotoxic activity *in vitro* against tumor cells and then *in vivo* in SCID mice that have received tumor cells displaying the relevant TAA.

Claims

1. A method to prepare an isolated nucleic acid molecule comprising a nucleotide sequence encoding at least one of the variable regions of the α and β chains of a non-human TCR which TCR is human HLA-restricted and specific for a tumor-associated antigen, which method comprises

cloning or amplifying a nucleic acid molecule containing said encoding nucleotide sequence from cytotoxic T lymphocytes (CTL) prepared by a method which comprises

immunizing a transgenic non-human vertebrate which is modified so as to express at least one human HLA antigen with said tumor-associated antigen (TAA) so as to effect the production in said mouse of cytotoxic T lymphocytes which display human HLA-restricted TCR specific for said TAA and which contain nucleic acid molecules comprising nucleotide sequences encoding said variable regions of the α and β chains of said TCR, and

recovering said CTL.

2. The method of claim 1 wherein said HLA antigen is a A2.

3. The method of claim 1 wherein said non-human vertebrate is a mouse.

4. The method of claim 3 wherein said amplifying is effected by a polymerase chain reaction using primers derived from murine TCR.

5. The method of claim 4 wherein said primers are essentially as set forth in Figure 6.

6. An isolated nucleic acid molecule which comprises a nucleotide sequence encoding a variable region of a non-human TCR α or β peptide wherein said TCR is human HLA-restricted and specific for a tumor-associated antigen.

7. The nucleic acid molecule of claim 6 which comprises the α or β variable region of the said TCR fused to the ζ region of CD3, CD8 or CD16.

8. The nucleic acid molecule of claim 7 wherein said ζ region is that of human CD3, CD8 or CD16.

9. The nucleic acid molecule wherein said non-human TCR is murine.

10. The nucleic acid molecule of claim 6 wherein said nucleotide sequence encodes a single-chain TCR.

11. The nucleic acid molecule of claim 10 wherein said single-chain TCR consists of the variable α region fused to variable β region by a flexible linker and said β region is fused to a ζ region.

12. The nucleic acid molecule of claim 11 wherein said flexible linker is of the formula $(Gly_4Ser_3)_3$.

13. The nucleic acid molecule of claim 11 wherein said ζ chain is that of CD3, CD8 or CD16.

14. The nucleic acid molecule of claim 13 wherein the ζ chain is derived from human CD3, CD8 or CD16.

15. A recombinant expression system which expression system comprises the nucleotide sequence of claim 6 operatively linked to control sequences for effecting its expression in a host cell.

16. A recombinant host cell modified to contain the expression system of claim 15.

17. The recombinant cells of claim 16 which are T cells.

18. A method to obtain cells which display TCR or a functional derivative thereof at their surface, said TCR or derivative being human HLA-restricted and specific for a tumor-associated antigen, which method comprises culturing the cells of claim 16 under conditions wherein said nucleotide sequence is expressed and said TCR or derivative is displayed at the surface.

19. Recombinant cells displaying a TCR receptor or derivative thereof at their surface wherein said TCR or derivative is human HLA-restricted and specific for a tumor-associated antigen prepared by the method of claim 18.

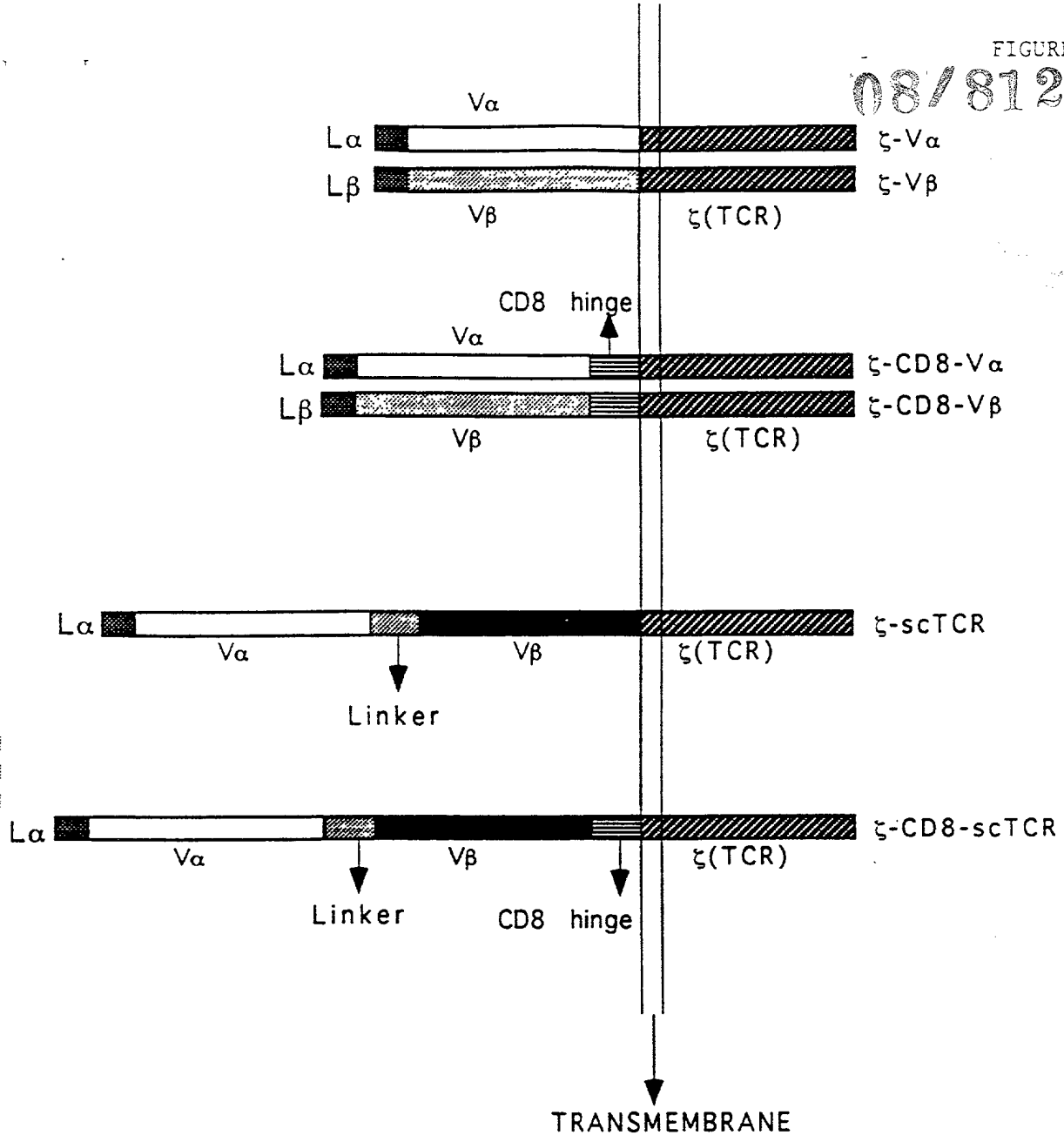
20. A method to identify antigens associated with a tumor which method comprises contacting said tumor or a fraction thereof with the cells of claim 19 under conditions wherein said tumor or fraction is lysed only if said tumor displays the TAA for which said TCR or derivative is specific.

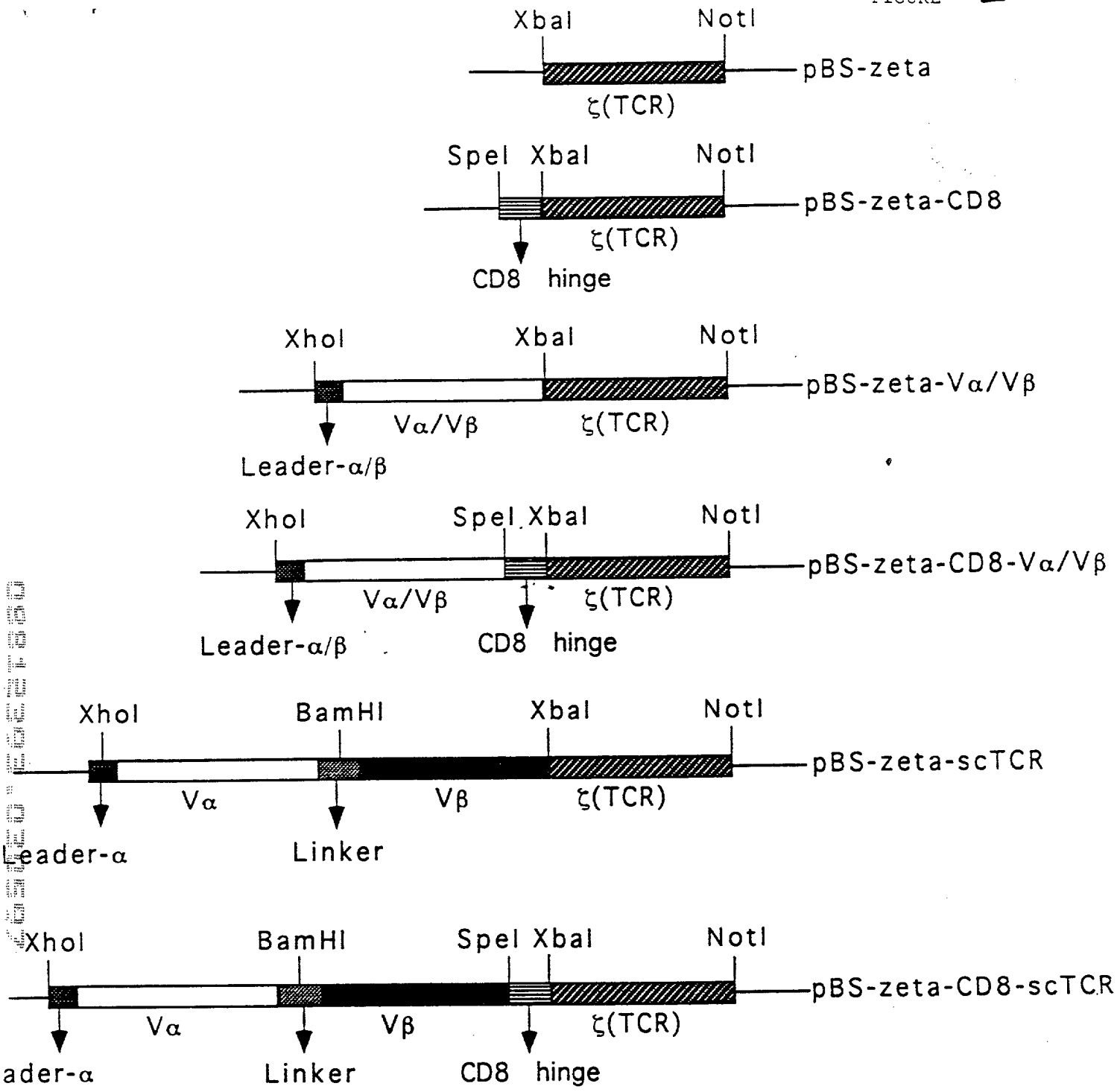
21. A method to effect treatment of a tumor in a human, wherein said tumor is characterized by a specific tumor-associated antigen (TAA) which method comprises administering to said human subject peripheral blood cells from said subject which have

been modified to contain an expression system for a nucleotide sequence which encodes a TCR or derivative thereof which is human HLA-restricted and specific for said TAA.

Abstract

Methods are described to obtain nucleic acid molecules that encode T cell receptors and their derivatives that are human HLA-restricted and which are specific for tumor-associated antigens found in human tumors. These nucleic acids are useful in preparing recombinant cells for diagnosis and therapy of human tumors.




XbaI
NotI
pBS-zeta
ζ(TCR)
SpeI
XbaI
NotI
pBS-zeta-CD8
ζ(TCR)
CD8 hinge
XhoI
XbaI
NotI
pBS-zeta-Vα/Vβ
Vα/Vβ
ζ(TCR)
Leader-α/β
XhoI
SpeI
XbaI
NotI
pBS-zeta-CD8-Vα/Vβ
Vα/Vβ
ζ(TCR)
Leader-α/β
CD8 hinge
XhoI
BamHI
XbaI
NotI
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Vα
Vβ
ζ(TCR)
Leader-α
Linker
XhoI
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SpeI
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Vα
Vβ
ζ(TCR)
ader-α
Linker
CD8 hinge

Name : seq-z88c-1
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n Table : Universal

08/812393

FIGURE 3A

LA

XhoI

```
          9          18          27          36          45          54
CTC GAG ATG CAG AGG AAC CTG GGA GCT GTG CTG GGG ATT CTG TGG GTG CAG ATT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   E   M   Q   R   N   L   G   A   V   L   G   I   L   W   V   Q   I

         63          72          81          90          99         108
TGC TGG CTG AAA GAA CAG CAA GTG CAG CAG AGT CCC GCA TCC TTG GTT CTG CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   W   L   K   E   Q   Q   V   Q   Q   S   P   A   S   L   V   L   Q

        117         126         135         144         153         162
GAG GGG GAG AAC GCA GAG CTC CAG TGT AGC TTT TCC ATC TTT ACA AAC CAG GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 E   G   E   N   A   E   L   Q   C   S   F   S   I   F   T   N   Q   V

        171         180         189         198         207         216
CAG TGG TTT TAC CAA CGT CCT GGG GGA AGA CTC GTC AGC CTG TTG TAC AAT CCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   W   F   Y   Q   R   P   G   G   R   L   V   S   L   L   Y   N   P

        225         234         243         252         261         270
TCT GGG ACA AAG CAG AGT GGG AGA CTG ACA TCC ACA ACA GTC ATT AAA GAA CGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   G   T   K   Q   S   G   R   L   T   S   T   T   V   I   K   E   R

        279         288         297         306         315         324
TGC AGC TCT TTG CAC ATT TCC TCC TCC CAG ATC ACA GAC TCA GGC ACT TAT CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   S   S   L   H   I   S   S   S   Q   I   T   D   S   G   T   Y   L

        333         342         351         360         369         378
TGT GCC TCA AAT TCT GGA GGA AGC AAT GCA AAG CTA ACC TTC GGG AAA GGC ACT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   A   S   N   S   G   G   S   N   A   K   L   T   F   G   K   G   T

        387         396         405         414         423         432
AAA CTC TCT GTT AAA TCA GGT GGC GGA GGG TCT GGC GGG GGT GGA TCC GGG GGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   L   S   V   K   S   G   G   G   G   S   G   G   G   G   S   G   G
                                                  BamHI
                                                  LINKER

        441         450         459         468         477         486
GGA GGC TCA GAG GCT GCA GTC ACC CAA AGC CCA AGA AAC AAG GTG GCA GTA ACA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   G   S   E   A   A   V   T   Q   S   P   R   N   K   V   A   V   T

        495         504         513         522         531         540
GGA GGA AAG GTG ACA TTG AGC TGT AAT CAG ACT AAT AAC CAC AAC AAC ATG TAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   G   K   V   T   L   S   C   N   Q   T   N   N   H   N   N   M   Y

        549         558         567         576         585         594
TGG TAT CGG CAG GAC ACG GGG CAT GGG CTG AGG CTG ATC CAT TAT TCA TAT GGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 W   Y   R   Q   D   T   G   H   G   L   R   L   I   H   Y   S   Y   G

        603         612         621         630         639         648
GCT GGC AGC ACT GAG AAA GGA GAT ATC CCT GAT GGA TAC AAG GCC TCC AGA CCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   G   S   T   E   K   G   D   I   P   D   G   Y   K   A   S   R   P
```

VL

VH

```
          657        666        675        684        693        702
AGC CAA GAG AAC TTC TCC CTC ATT CTG GAG TTG GCT ACC CCC TCT CAG ACA TCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
S   Q   E   N   F   S   L   I   L   E   L   A   T   P   S   Q   T   S

          711        720        729        738        747        756
GTG TAC TTC TGT GCC AGC GGT GAG ACA GGG ACC AAC GAA AGA TTA TTT TTC GGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
V   Y   F   C   A   S   G   E   T   G   T   N   E   R   L   F   F   G

          765        774        783 SpE    792        801        810
CAT GGA ACC AAG CTG TCT GTC CTG ACT AGT AAC TCC ATC ATG TAC TTC AGC CAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H   G   T   K   L   S   V   L   T   S   N   S   I   M   Y   F   S   H

          819        828        837        846        855        864
TTC GTG CCG GTC TTC CTG CCA GCG AAG CCC ACC ACG ACG CCA GCG CCG CGA CCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
F   V   P   V   F   L   P   A   K   P   T   T   T   P   A   P   R   P

          873        882        891        900        909        918
CCA ACA CCG GCG CCC ACC ATC GCG TCG CAG CCC CTG TCC CTG CGC CCA TCT AGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
P   T   P   A   P   T   I   A   S   Q   P   L   S   L   R   P   S   S

 Ab CH  927        936        945        954        963        972
TCT AGA GAT CCC AAA CTC TGC TAC CTG CTG GAT GGA ATC CTC TTC ATC TAT GGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
S   R   D   P   K   L   C   Y   L   L   D   G   I   L   F   I   Y   G

          981        990        999       1008       1017       1026
GTC ATT CTC ACT GCC TTG TTC CTG AGA GTG AAG TTC AGC AGG AGC GCA GAC GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
V   I   L   T   A   L   F   L   R   V   K   F   S   R   S   A   D   A

         1035       1044       1053       1062       1071       1080
GCC GCG TAC CAG CAG GGC CAG AAC CAG CTC TAT AAC GAG CTC AAT CTA GGA CGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
P   A   Y   Q   Q   G   Q   N   Q   L   Y   N   E   L   N   L   G   R

         1089       1098       1107       1116       1125       1134
AGA GAG GAG TAC GAT GTT TTG GAC AAG AGA CGT GGC CGG GAC CCT GAG ATG GGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
R   E   E   Y   D   V   L   D   K   R   R   G   R   D   P   E   M   G

         1143       1152       1161       1170       1179       1188
GGA AAG CCG AGA AGG AAG AAC CCT CAG GAA GGC CTG TAC AAT GAA CTG CAG AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
G   K   P   R   R   K   N   P   Q   E   G   L   Y   N   E   L   Q   K

         1197       1206       1215       1224       1233       1242
GAT AAG ATG GCG GAG GCC TAC AGT GAG ATT GGG ATG AAA GGC GAG CGC CGG AGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
D   K   M   A   E   A   Y   S   E   I   G   M   K   G   E   R   R   R

         1251       1260       1269       1278       1287       1296
GGC AAG GGG CAC GAT GGC CTT TAC CAG GGT CTC AGT ACA GCC ACC AAG GAC ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
G   K   G   H   D   G   L   Y   Q   G   L   S   T   A   T   K   D   T

         1305       1314       1323       1332  NotI 1341    1350
TAC GAC GCC CTT CAC ATG CAG GCC CTG CCC CCT CGC TAA GCG GCC GCC ACC GCG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Y   D   A   L   H   M   Q   A   L   P   P   R   *   A   A   A   T   A
```

CDS HINGE

2 CHAIN.

STOP

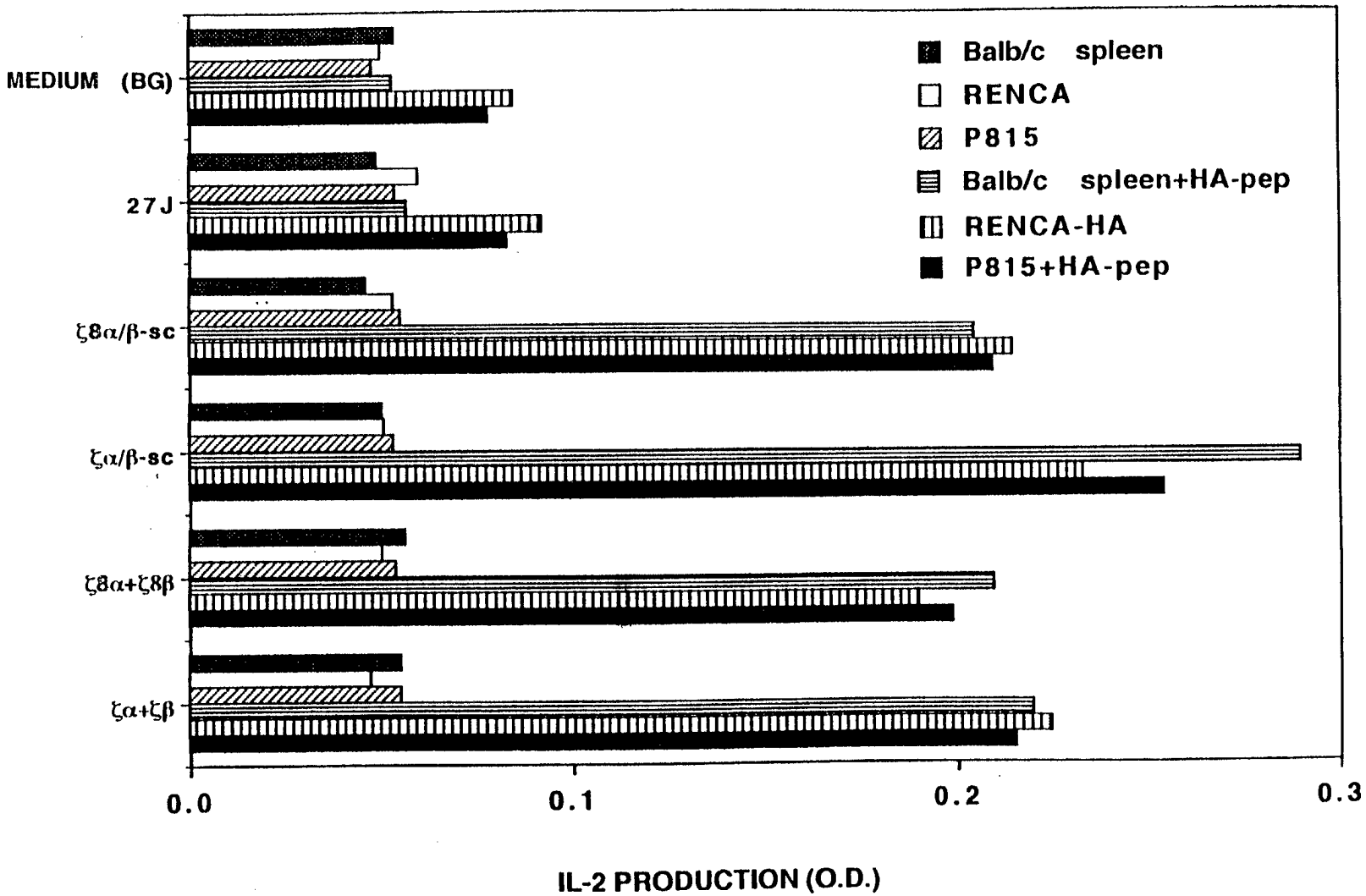

FIGURE 4

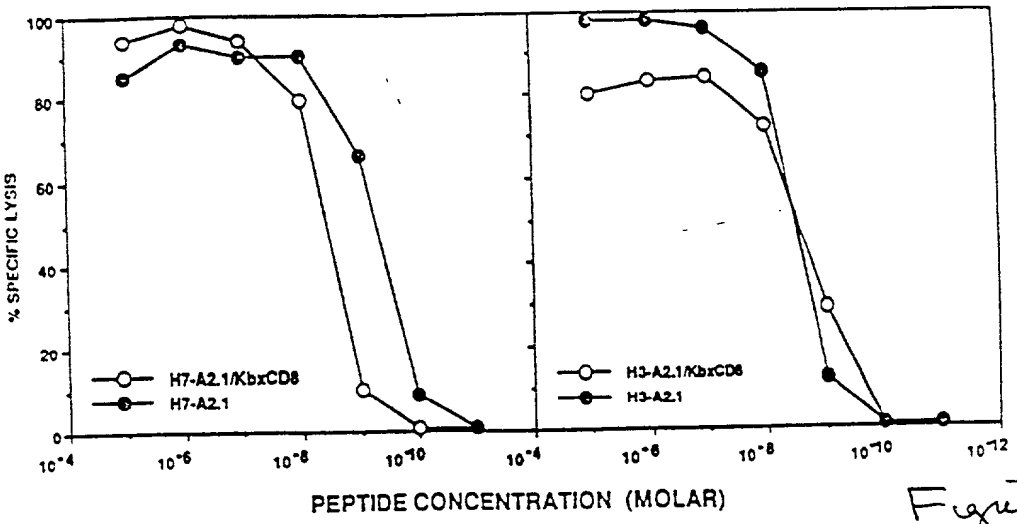

Figure 5

Alpha Groups

1.

Vα1	CCC AAG GCA CTG ATG TTC ATC TTC
Vα2	TGA GAC AAA GTC CCC AAT CTC TGA CAG
Vα3	CTG CAG CTG CTC CTC AAG TAC TAT TC
Vα4.1,2,3	TCC CGG AGA AGG TCC ACA GTT CCT CTT T
Vα4 4	GAA GCA GCA GAG GGT TTG AAG CCA CAT AC

2.

Vα5	GGC AGG TCT TCA GTT GCT TAT GAA GGT
Vα6	GGT TCC TCT TCA GGG TCC AGA ATA TGT
Vα7	GCG AAG AAC TCA CCC TGG ACT GTT CAT
Vα8	GAG CTC CAC AGA CAA CAA GAG GAC CGA GCA
Vα9	GAG CTG CGA CGT TCC TTA GTG ACT GTG

3.

Vα10	CCT CGT CAG CCT GTT GTC CAA TCC TTC TGG
Vα11	CAG CCT CAT CAA TCT GTT CTA CTT GGC T
Vα12	CCA CCA GGG ACC ACA GTT TAT CAT TCA A
Vα14	ACC TGG AGA GAA TCC TAA GCT CAT CAT
Vα15	AGG TCT TGT GTC CCT GAC AGT CCT GGT T

4.

Vα16	CAA GCA AAC ACT GTA GTG CAG AGC CCT TCC
Vα17	CAA GAC ATC CAT AAC TGC CCT ACA G
Vα18	GTG TAT GAA ACC CAG GAC AGT TCT TAC
Vα19	CCG TAT TTC TTT CTT ATG TTG TTT TGG AT
Vα20	CAA AGC TCT CCA TCG CTG ACT GTT CAA G

Beta Groups

1.

Vβ1	ATC TAA TCC TGG GAA GAG CAA AT
Vβ2	GGC GTC TGG TAC CAC GTG GTC AA
Vβ3	GTG AAA GGG CAA GGA CAA AAA GC
Vβ4	GAT ATG CGA ACA GTA TCT AGG C
Vβ5.1	ACA TAA TCA AAG GAA AGG GAG AA

2.

Vβ6	TCC TGA TTG GTC AGG AAG GGC AA
Vβ7	TAC CTG ATC AAA AGA ATG GGA GA
Vβ8.1	ATA ACC ATG ACA ATA TGT ACT GG
Vβ8.2	ATA ACC ACA ACA ACA TGT ACT GG
Vβ8.3	ATA GCC ACA ACT ACA TGT ACT GG

3.

Vβ9	AGC TTG CAA GAG TTG GAA AAC CA
Vβ10	GAT TAT GTT TAG CTA CAA TAA TA
Vβ11	ACA AGG TGA CAG GGA AGG GAC AA
Vβ12	ACC TAC AGA ACC CAA GGA CTC AG
Vβ13	CAG TTG CCC TCG GAT CGA TTT TC

4.

Vβ14	GCC GAG ATC AAG GCT GTG GGC AG
Vβ15	AGA ACC ATC TGT AAG AGT GGA AC
Vβ16	CAT CAA ATA ATA GAT ATG GGG CA
Vβ17	GTA GTC CTG AAA AAG GGC ACA CT
Vβ18	CAT CTG TCA AAG TGG CAC TTC A

ile Name : SEQ-H7-ALPHA-1
ange : 1 - 393 Mode : Normal
odon Table : Universal


```
          9          18          27          36          45          54
ATG AAA TCC TTG AGT GTT TCC CTA GTG GTC CTG TGG CTC CAG TTA AAC TGG GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Met Lys Ser Leu Ser Val Ser Leu Val Val Leu Trp Leu Gln Leu Asn Trp Val

         63          72          81          90          99         108
CAG AGC CAG CAG AAG GTG CAG CAG AGC CCA GAA TCC CTC AGT GTC CCA GAG GGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gln Ser Gln Gln Lys Val Gln Gln Ser Pro Glu Ser Leu Ser Val Pro Glu Gly

        117         126         135         144         153         162
GGC ATG GCC TCT CTC AAC TGC ACT TCA AGT GAT CGC AAT TTT CAG TAT TTC TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Met Ala Ser Leu Asn Cys Thr Ser Ser Asp Arg Asn Phe Gln Tyr Phe Trp

        171         180         189         198         207         216
TGG TAC AGA CAG CAT TCT GGA GAA GGC CCC AAA GCA CTG ATG TCC ATC TTC TCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Trp Tyr Arg Gln His Ser Gly Glu Gly Pro Lys Ala Leu Met Ser Ile Phe Ser

        225         234         243         252         261         270
GAT GGT GAC AAG AAA GAA GGC AGA TTC ACA GCT CAC CTC AAT AAG GCC AGC CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Gly Asp Lys Lys Glu Gly Arg Phe Thr Ala His Leu Asn Lys Ala Ser Leu

        279         288         297         306         315         324
CAT GTT TCC CTG CAC ATC AGA GAC TCC CAG CCC AGT GAC TCC GCT CTC TAC TTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
His Val Ser Leu His Ile Arg Asp Ser Gln Pro Ser Asp Ser Ala Leu Tyr Phe

        333         342         351         360         369         378
TGT GCA GTT ATG GAT TAT AAC CAG GGG AAG CTT ATC TTT GGG CAG GGT ACC AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Cys Ala Val Met Asp Tyr Asn Gln Gly Lys Leu Ile Phe Gly Gln Gly Thr Lys

        387
TTA TCT ATC AAG CCC 3'
--- --- --- --- ---
Leu Ser Ile Lys Pro
```

File Name : h7-beta
Range : 1 - 402 Mode : Normal
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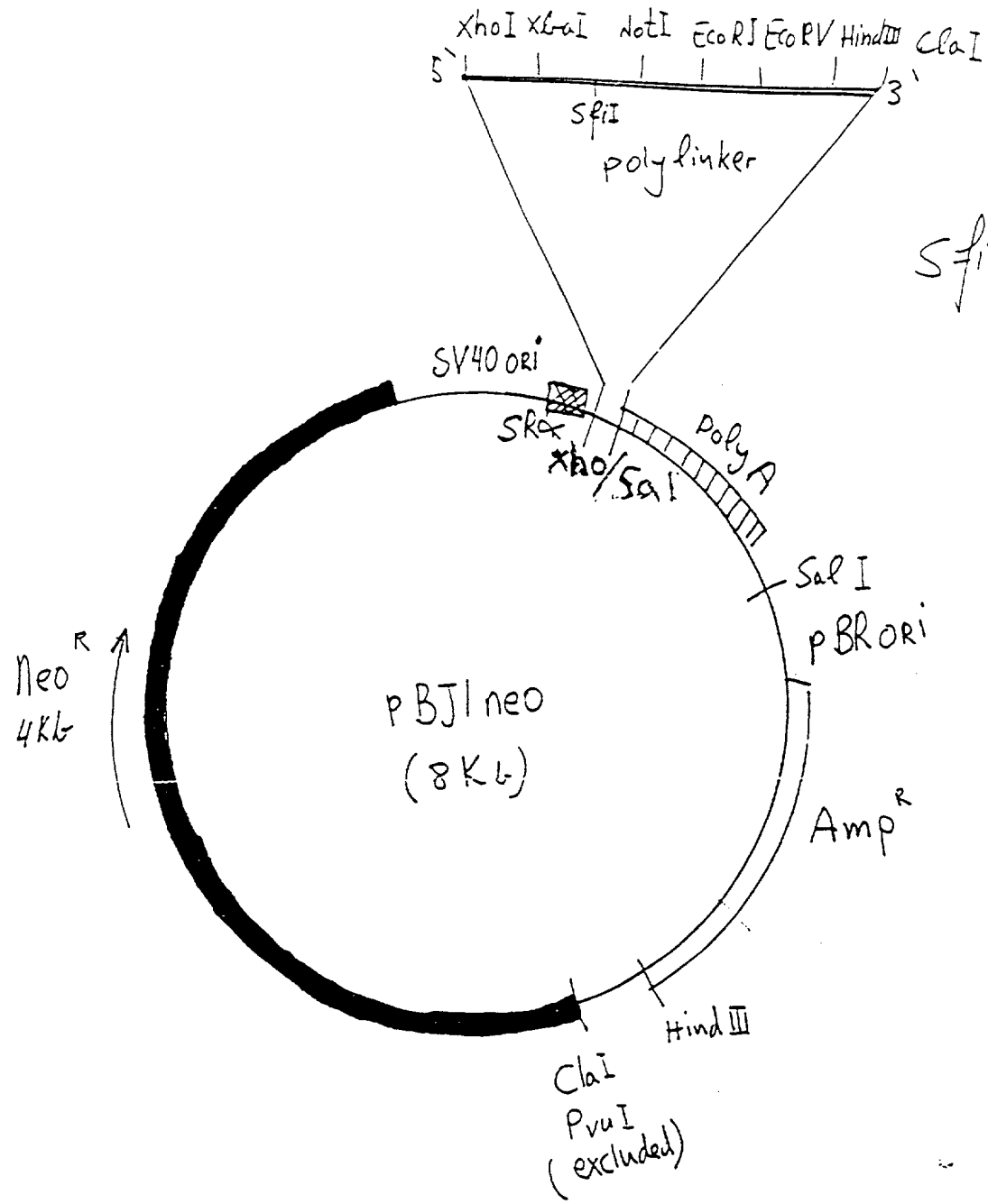
FIG 7B

08/812393

```
            9          18          27          36          45          54
5' ATG GGC TCC AGA CTC TTC TTT GTG GTT TTG ATT CTC CTG TGT GCA AAA CAC ATG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Met Gly Ser Arg Leu Phe Phe Val Val Leu Ile Leu Leu Cys Ala Lys His Met

           63          72          81          90          99         108
   GAG GCT GCA GTC ACC CAA AGT CCA AGA AGC AAG GTG GCA GTA ACA GGA GGA AAG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Glu Ala Ala Val Thr Gln Ser Pro Arg Ser Lys Val Ala Val Thr Gly Gly Lys

          117         126         135         144         153         162
   GTG ACA TTG AGC TGT CAC CAG ACT AAT AAC CAT GAC TAT ATG TAC TGG TAT CGG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Val Thr Leu Ser Cys His Gln Thr Asn Asn His Asp Tyr Met Tyr Trp Tyr Arg

          171         180         189         198         207         216
   CAG GAC ACG GGG CAT GGG CTG AGG CTG ATC CAT TAC TCA TAT GTC GCT GAC AGC
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Gln Asp Thr Gly His Gly Leu Arg Leu Ile His Tyr Ser Tyr Val Ala Asp Ser

          225         234         243         252         261         270
   ACG GAG AAA GGA GAT ATC CCT GAT GGG TAC AAG GCC TCC AGA CCA AGC CAA GAG
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Thr Glu Lys Gly Asp Ile Pro Asp Gly Tyr Lys Ala Ser Arg Pro Ser Gln Glu

          279         288         297         306         315         324
   AAT TTC TCT CTC ATT CTG GAG TTG GCT TCC CTT TCT CAG TCA GCT GTA TAT TTC
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Asn Phe Ser Leu Ile Leu Glu Leu Ala Ser Leu Ser Gln Ser Ala Val Tyr Phe

          333         342         351         360         369         378
   TGT GCC AGC AGC GAT TTC GCC GGG ACA GGG GGC TTC TAT GAA CAG TAC TTC GGT
   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
   Cys Ala Ser Ser Asp Phe Ala Gly Thr Gly Gly Phe Tyr Glu Gln Tyr Phe Gly

          387         396
   CCC GGC ACC AGG CTC ACG GTT TCT 3'
   --- --- --- --- --- --- --- ---
   Pro Gly Thr Arg Leu Thr Val Ser
```

FIGURE 8



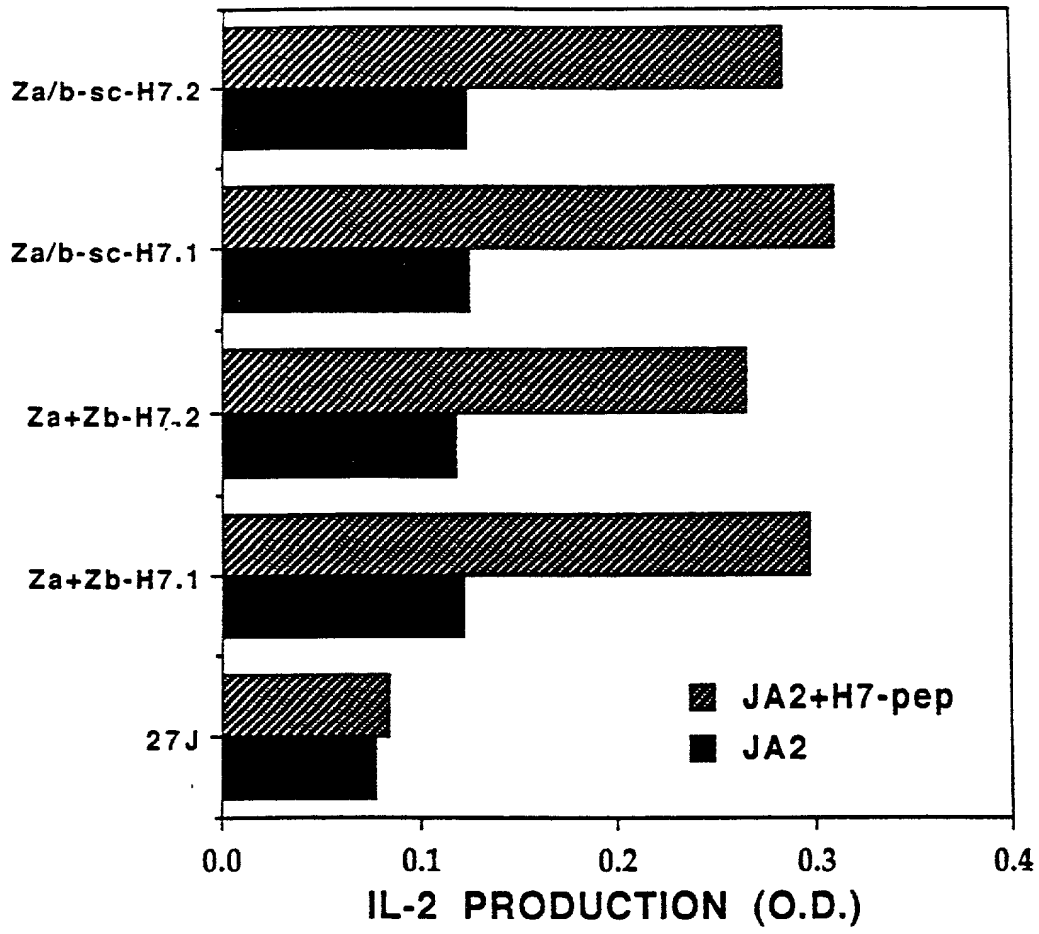
SfiI - notun

Ref:

pBJIneo - MCB 8: 466, 1988

Polylinker - Science, 249: 677, 1990

FIGURE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0.3
0.4
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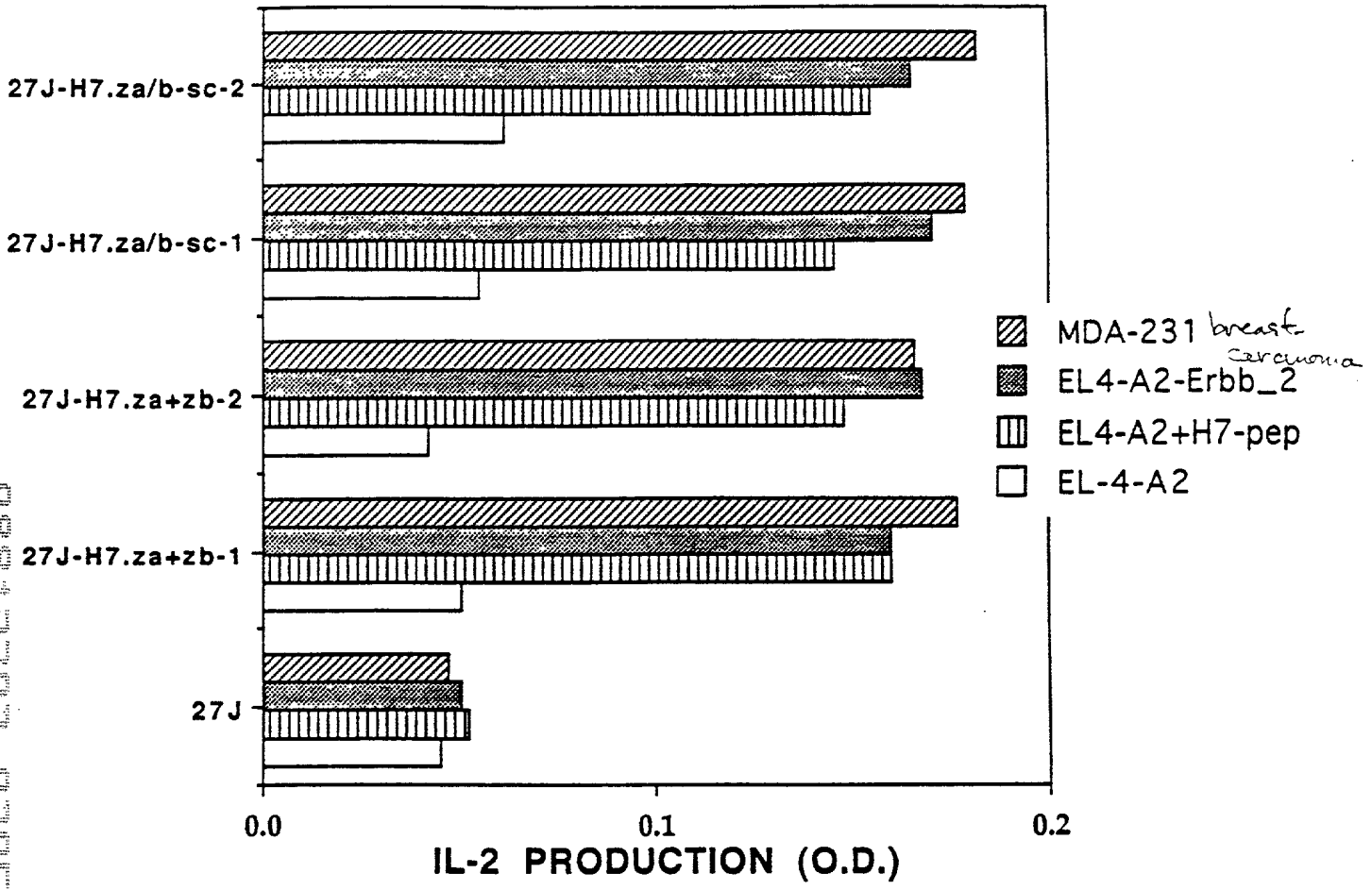

Applicant/Patentee: Linda Sherman et al.
Serial No./Patent No.:
Filed on/Issued : 3 March 1997
For: RECOMBINANT CONSTRUCTS ENCODING T CELL RECEPTORS SPECIFIC FOR HUMAN HLA-RESTRICTED TUMOR ANTIGENS

Docket No.: 313332000100
Client Reference: SUN 0001P

VERIFIED STATEMENT CLAIMING SMALL ENTITY STATUS
37 C.F.R. §§ 1.9(f) AND 1.27(c) -- SMALL BUSINESS CONCERN

I hereby declare that I am

☐ the owner of the small business concern identified below:
☒ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN: The Scripps Research Institute

ADDRESS OF CONCERN: 10550 North Torrey Pines Road, La Jolla, California 92037

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 C.F.R. § 121.12, and reproduced in 37 C.F.R. § 1.9(d), for purposes of paying reduced fees to the United States Patent and Trademark Office, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled RECOMBINANT CONSTRUCTS ENCODING T CELL RECEPTORS SPECIFIC FOR HUMAN HLA-RESTRICTED TUMOR ANTIGENS by inventor(s) Linda A. Sherman and Joseph Lustgarten
described in

☐ the specification filed herewith with title as listed above.
☒ the application identified above.
☐ the patent identified above.

If the rights held by the above identified business concern are not exclusive, each individual, concern or organization having rights in the invention must file separate verified statements averring to their status as small entities, and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 C.F.R. § 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 C.F.R. § 1.9(d), or a nonprofit organization under 37 C.F.R. § 1.9(e).

Each person, concern or organization having any rights in the invention is listed below:

☐ no such person, concern, or organization exists.
☒ each such person, concern or organization is listed below.

NAME	ADDRESS	TYPE
Sunol Molecular Corporaion	2173 N.W. 99th Avenue MS W700 Miami, Florida 33172	☐ Individual ☐ Small Business Concern ☒ Nonprofit Organization

Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 C.F.R. § 1.27)

I acknowledge the duty to file, in this application or patent, notification or any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. § 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Douglas A. Bingham
TITLE OF PERSON IF OTHER THAN OWNER: Vice President and General Counsel
ADDRESS OF PERSON SIGNING: 10550 North Torrey Pines Road, La Jolla, California 92037

SIGNATURE: _____ DATE: 18 Apr 97

PTO/SB/10 (10-92)
dc-68800

PATENT
Docket No. 313332000100
Client Ref. SUN 0001P

#3

COMBINED DECLARATION AND POWER OF ATTORNEY FOR UTILITY PATENT APPLICATION

AS A BELOW-NAMED INVENTOR, I HEREBY DECLARE THAT:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: RECOMBINANT CONSTRUCTS ENCODING T CELL RECEPTORS SPECIFIC FOR HUMAN HLA-RESTRICTED TUMOR ANTIGENS, the specification of which is attached hereto unless the following box is checked:

☒ was filed on 5 March 1997 as United States Application Serial No.

I HEREBY STATE THAT I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE.

I acknowledge the duty to disclose information which is material to the patentability as defined in 37 C.F.R. § 1.56.

I hereby claim benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

Application Serial No.	Filing Date
60/012,845	5 March 1996

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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